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Application of a Novel Radioimmunoassay to Identify Baculovirus Structural Proteins that Share Interspecies Antigenic Determinants

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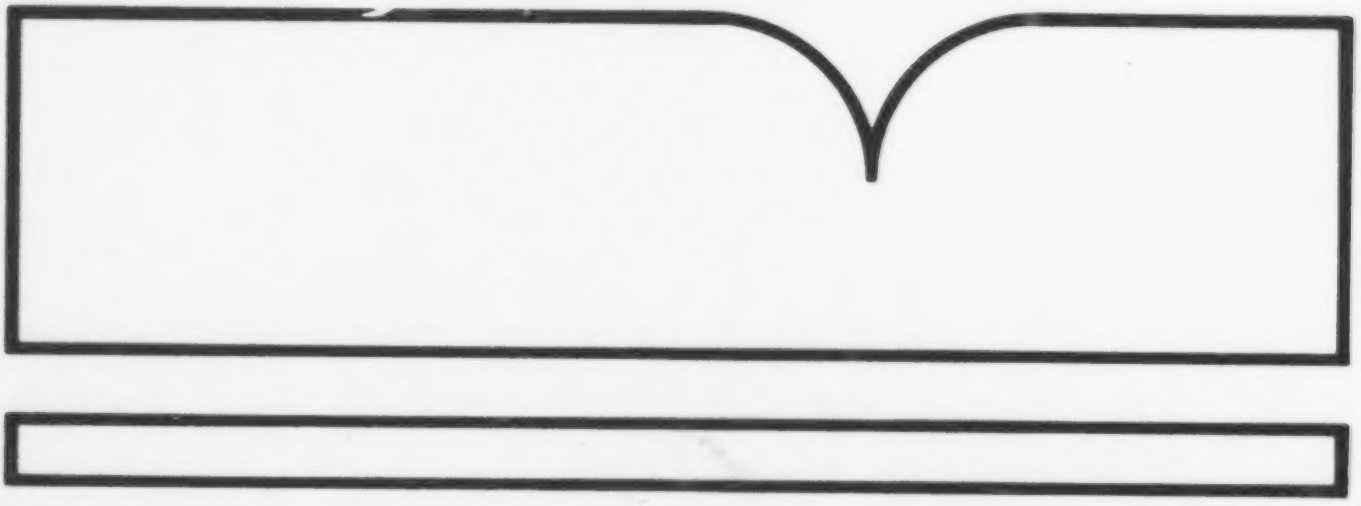

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16. ABSTRACT
Viral proteins were electrophoresed in polyacrylamide gels, transferred to nitrocellulose, and incubated with viral antisera, and the antibodies were detected with ^{125}I-labeled *Staphylococcus aureus* protein A. Antisera were prepared to purified and intact virions from five baculoviruses: *Autographa californica*, *Porthetria dispar*, *Trichoplusia ni*, and *Heliothis zea* nuclear polyhedrosis viruses (NPVs) and *T. ni* granulosis virus (GV). These antisera were tested against the virion structural polypeptides of 17 different species of baculoviruses. Specific multiple-nucleocapsid NPV (MNPV), single-nucleocapsid NPV (SNPV), and GV virion polypeptides were shown to have similar antigenic determinants and thus be immunologically related. The molecular weights of the virion polypeptides with cross-reacting antigenic determinants were identified. Antisera prepared to purified A. *californica* and H. *zea* MNPV polyhedrin (the occlusion body protein from NPVs) recognized antigenic determinants on all the polyhedrins and granulins (occlusion body protein from GVs) that were tested. No immunological relationship was detected between A. *californica* MNPV polyhedrin and any of the A. *californica* MNPV virion structural polypeptides present on either the virus isolated from occlusion bodies or A. *californica* MNPV extracellular virus from infected-cell cultures.

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Application of a Novel Radioimmunoassay to Identify Baculovirus Structural Proteins That Share Interspecies Antigenic Determinants

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Immunological comparisons were made of baculovirus structural proteins by using a modification of the radioimmunological techniques described by Renart et al. (Proc. Natl. Acad. Sci. U.S.A. **76:** 3116–3120, 1979) and Towbin et al. (Proc. Natl. Acad. Sci. U.S.A. **76:** 4350–4354, 1979). Viral proteins were electrophoresed in polyacrylamide gels, transferred to nitrocellulose, and incubated with viral antisera, and the antibodies were detected with ^{125}I-labeled *Staphylococcus aureus* protein A. Antisera were prepared to purified and intact virions from five baculoviruses: *Autographa californica, Porthetria dispar, Trichoplusia ni,* and *Heliothis zea* nuclear polyhedrosis viruses (NPVs) and *T. ni* granulosis virus (GV). These antisera were tested against the virion structural polypeptides of 17 different species of baculoviruses. Specific multiple-nucleocapsid NPV (MNPV), single-nucleocapsid NPV (SNPV), and GV virion polypeptides were shown to have similar antigenic determinants and thus be immunologically related. The molecular weights of the virion polypeptides with cross-reacting antigenic determinants were identified. Antisera prepared to purified *A. californica* and *H. zea* MNPV polyhedrin (the occlusion body protein from NPVs) recognized antigenic determinants on all the polyhedrins and granulins (occlusion body protein from GVs) that were tested. No immunological relationship was detected between *A. californica* MNPV polyhedrin and any of the *A. californica* MNPV virion structural polypeptides present on either the virus isolated from occlusion bodies or *A. californica* MNPV extracellular virus from infected-cell cultures.

Baculoviruses, a group of enveloped double-stranded DNA animal viruses in the family Baculoviridae (12), are etiologically involved in naturally occurring infections in many species spanning seven orders of insects. Different baculovirus species have been classified into one of three subgroups according to morphological criteria. Subgroup A viruses, the nuclear polyhedrosis viruses (NPVs), have been isolated from more than 300 insect species and from crustaceans. The virions in this subgroup contain either a single nucleocapsid (SNPV) or many nucleocapsids (MNPV) per envelope. Both SNPVs and MNPVs form nuclear inclusions late in infection in which many virions are embedded per inclusion body. Subgroup B viruses, the granulosis viruses (GVs), have been isolated from about 50 species of lepidoptera. These viruses have one nucleocapsid per envelope and one virion per inclusion body. Subgroup C viruses, the nonoccluded viruses, are not compared in this study.

Nucleotide sequence homology studies and radioimmunoassays (RIAs) are two of the more sensitive methods used to demonstrate genetic relatedness among viruses and similar antigenic determinants in viral gene products, respectively. It is well established that conserved antigenic determinants are more readily detected than their corresponding nucleotide sequences (1, 21, 34). For example, a sensitive RIA was recently used to demonstrate an immunological linkage between retroviruses which had diverged to the point where base sequence homology could no longer be detected (5).

Many immunological studies of baculoviruses have been done with the major inclusion body protein, called polyhedrin if isolated from NPVs and granulin if isolated from GVs (22). These proteins are composed of a single polypeptide with a molecular weight of 25,000 to 33,000 (25). Polyhedrins and granulins have been shown to have a common antigenic determinant(s) by a variety of immunological techniques, including RIA (23) and enzyme immunoassay (4). In addition, tryptic peptide mapping studies (8, 11, 24) and direct amino acid sequence data (16) have shown that some of the primary sequences of the polyhedrins and granulins are similar.

The virions of NPVs and GVs are composed of approximately 15 to more than 30 polypeptides (3, 25, 30). The interspecies immunological relationships of these virion proteins is not yet well understood.

Recently, a simple and sensitive RIA technique was developed to detect specific antigenic determinants of polypeptides transferred from polyacrylamide electrophoresis gels to nitrocellulose filters (15, 27). For convenience, we will refer to this technique as protein blot RIA. Protein blot RIA has several advantages over other liquid- or solid-phase RIAs for the immunological analysis of baculovirus structural proteins: (i) virions are composed of many polypeptides that have different solubility properties in the nonionic detergents commonly used in liquid-phase RIAs (25), and if a particular antigen is a member of a complex aggregate of different polypeptides as a result of incomplete disruption of the virions or aggregation of virion polypeptides, the entire aggregate will be immunoprecipitated; (ii) proteolytic degradation of the polypeptides may be a problem during long incubation times; and (iii) immunological cross-reactions between baculovirus virion polypeptides may be due to contaminating polyhedrins and granulins or contaminating host or viral antigens. Protein blot RIA is an alternative approach to help circumvent such problems (15).

In this study, we have undertaken a survey to detect similar antigenic determinants among the structural polypeptides of NPVs and GVs. Using protein blot RIA, we identified the molecular weights of virion polypeptides that reacted with antibodies in antisera prepared against purified virions from five baculoviruses. Certain MNPV, SNPV, and GV virion polypeptides were shown to share related, cross-reacting antigenic determinants. In addition, polyhedrins and granulins from 16 baculoviruses were shown to be immunologically related.

MATERIALS AND METHODS

Viruses and cells. The continuous cell line of *Trichoplusia ni*, TN-368, was maintained as described previously (31). Table 1 shows the baculoviruses compared in this study and their sources.

Purification of viruses. Inclusion bodies were isolated from infected larvae as reported (25). To isolate enveloped nucleocapsids, the inclusion bodies were suspended at a concentration of 10 mg of protein per ml in distilled water and included at 70°C for 20 min (24). The heat-treated inclusion bodies were cooled on ice, disrupted by adding an equal volume of 0.05 M Na_2CO_3–0.05 M NaCl–0.002 M EDTA, pH 10.9, and incubated for 30 min on ice. The released virions were then purified by sucrose density gradient centrifugation as described (25).

Autographa californica MNPV (AcMNPV) extracellular virus (ECV) was purified from the medium of infected TN-368 cells at 48 h postinfection as described (18).

Preparation of polyhedrin and granulin. The inclusion bodies were purified and disrupted in alkali as described above. The alkaline-soluble polyhedrins and granulins were recovered from the top of sucrose gradients (density range of 1.15 to 1.27 g/ml) after centrifugation at 100,000 × *g* for 1 h and stored at −70°C.

Preparation of antisera. New Zealand white rabbits were injected intramuscularly with a solution containing 75 μg of intact AcMNPV, *Porthetria dispar* MNPV (PdMNPV), *T. ni* SNPV (TnSNPV), *Heliothis zea* SNPV (HzSNPV), or *T. ni* GV (TnGV) virions and an equal volume of Freund complete adjuvant. AcMNPV and HzSNPV polyhedrins were purified by two cycles of preparative sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (23) and checked for purity by analytical SDS-PAGE. Seventy-five micrograms of purified polyhedrin in 0.5 ml was mixed with 0.5 ml of Freund complete adjuvant and injected intramuscularly. Booster injections were given 1, 2, and 3 weeks later with equivalent concentrations of protein mixed with an equal volume of Freund incomplete adjuvant. One week after the last booster injection, the rabbits were bled.

PAGE. Electrophoresis in 10% polyacrylamide vertical slab gels was done by using the gel system of Laemmli (9) as described by Summers and Smith (25). The method of Weber and Osborn (33) was used to measure the apparent molecular weights of viral polypeptides as compared with molecular weight standards (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.). Carbonic anhydrase, a molecular weight standard of 30,000, has an apparent molecular weight which varies from 30,000 to 34,000 in our gel system. We have not used this protein for molecular weight determinations. The molecular weights of some of the virus polypeptides presented in this report are slightly different from those reported previously (25) due to a different set of protein standards used.

^{125}I-labeled protein A. Protein A from *Staphylococcus aureus* (Pharmacia) was iodinated to a specific activity of 2.5 to 5.0 μCi/μg by a modification of the chloramine-T method (7) as described by Marier et al. (10).

Partial renaturation and transfer of polypeptides to nitrocellulose filters. Virion polypeptides were transferred from SDS-polyacrylamide slab gels to nitrocellulose filters (Schleicher & Schuell Co., Keene, N. H.; BA85) essentially as described by Bowen et al. (2). After electrophoresis, the slab gel (14 by 24 by 0.15 cm) was placed in 200 ml of protein transfer buffer (10 mM Tris, pH 7.5–50 mM NaCl–2 mM EDTA–0.1 mM dithiothreitol) plus 4 M urea and gently agitated for 1 h. Next, 300 ml of fresh protein transfer buffer plus 4 M urea was added for an additional 3 h. This step removes most of the SDS from the gel and facilitates renaturation of the proteins (2).

The partially renatured proteins were transferred for 36 h at 37°C from the polyacrylamide gel onto two nitrocellulose filters by the protein blot method described by Bowen et al. (2). After transfer, the polyacrylamide gel was stained with Coomassie brilliant blue (25) to monitor the efficiency of removal of poly-

TABLE 1. *Baculovirus isolates*

Strain (abbreviation)	Geographic origin		Year isolated	Laboratory passage hosts	Source
	Country	Locality			
Autographa californica (AcMNPV)	U.S.A	Irvine, Calif.	1967	*T. ni*	P. V. Vail U.S. Dept. of Agriculture Fresno, Calif.
Rachiplusia ou (RoMNPV)	U.S.A.	Newland, Ind.	1960	*T. ni*	J. D. Paschke Dept. of Entomology Purdue University Layfayette, Ind.
Anticarsa gemmatalis (AgMNPV)	Brazil	Holambra I, Sao Paulo	1973	*A. gemmatalis*	G. E. Allen Dept. of Entomology University of Florida Gainesville, Fla.
Spodoptera frugiperda (SfMNPV)	U.S.A.	Tifton, Ga.	1966	*S. frugiperda*	J. J. Hamm U.S. Dept. of Agriculture Tifton, Ga.
Spodoptera exigua (SeMNPV)	U.S.A.	Geneva, County, Ala.	1977	*S. exigua*	J. J. Hamm
Orgyia pseudotsugata (OpMNPV)	U.S.A.			*O. pseudotsugata*	M. Martigononi U.S. Forestry Service U.S. Dept. of Agriculture Corvalis, Ore.
Porthetria dispar (PdMNPV)	U.S.A.	Bethany, Conn.	1969	*P. dispar*	F. Lewis U.S. Forestry Service U.S. Dept. of Agriculture Hamden, Conn.
Heliothis armiger (HaMNPV)	U.S.S.R.	Tadhzykistan	1974	*H. zea*	J. J. Hamm
Choristoneura fumiferana (CfMNPV)	Canada	Ontario	1951	*C. fumiferana*	B. Arif Insect Pathology Research Institute Sault Ste. Marie, Ontario, Canada
Pseudoletia separata (PsMNPV)	India	Karnatak		*P. separata*	S. V. Mathad Division of Entomology Karnatak University Karnatak, India
Trichoplusia ni (TnSNPV)	U.S.A.	Indiana	1962	*T. ni*	J. D. Paschke
Heliothis zea (HzSNPV)	U.S.A.	Elkar (trademarked product)		*H. zea*	J. J. Hamm
Pseudoplusia includens (PiSNPV)	Brazil	Londrina	1975	*T. ni*	G. E. Allen
Trichoplusia ni (TnGV)	U.S.A.	Indiana	1962	*T. ni*	J. D. Paschke
Spodoptera frugiperda (SfGV)	U.S.A.	Mississippi	1977	*S. frugiperda*	J. J. Hamm
Heliothis armiger (HaGV)	South Africa	Western Cape	1966	*H. zea*	J. J. Hamm
Plodia interpunctella (PiGV)	U.S.A.	Tifton, Ga.	1968	*P. interpunctella*	P. V. Vail

peptides from the gel. To aid in aligning virion polypeptides detected immunologically with those observed by Coomassie brilliant blue staining, some of the nitrocellulose filters containing transferred virion polypeptides were stained for 15 min with 0.1% aniline blue black in 43% methanol–10% acetic acid and destained for 1 h in 90% methanol–2% acetic acid.

Baculovirus virion preparations and protein standards were partially renatured in situ and then transferred from a 10% polyacrylamide gel (similar to that shown in Fig. 1) by the protein blot procedure to two nitrocellulose filters. One of the nitrocellulose filters was incubated as described below for the immunological detection of transferred proteins. Gelatin, antibody, and ^{125}I-labeled protein A were omitted from the buffers. Both the gel and the two nitrocellulose filters were stained for protein. Nearly all the protein had diffused from the gel with the exception of major polypeptides in which only trace quantities were left in the gel (not shown). Polypeptide patterns on the stained nitrocellulose filters were indistinguishable from those seen in Fig. 1, with the exception of some diffusion of the protein bands (not shown). These results were similar to those reported by Bowen et al. (2), which showed that polypeptides are transferred by the protein blot method from polyacrylamide gels to nitrocellulose filters both quantitatively and qualitatively and are efficiently retained on the filters during subsequent incubations.

RIA of polypeptides transferred from PAGE

gels. The following procedure is a modification of that described by Renart et al. (15). Incubations were done with the nitrocellulose filters sealed in polyethylene bags at 37°C, and the volumes of the solutions described below were those used for protein transfers made to 14 by 17-cm filters.

After transfer of partially renatured polypeptides to nitrocellulose filters, the filters were incubated in 50 mM Tris, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.25% (wt/vol) gelatin, and 0.05% (vol/vol) Nonidet P-40 (TEN-NP40 buffer) for 4 h. Next, filters were incubated for 12 to 18 h with 5 ml of antiserum diluted in TEN-NP40 buffer as described in each figure legend. To remove excess antibody, the filters were washed briefly with distilled water and then incubated in 25 ml of TEN-NP40 buffer for 2 h. ^{125}I-labeled protein A was diluted in TEN-NP40 buffer to 10^6 cpm/ml, and 5 ml was added to each filter and incubated for 12 h. To remove excess ^{125}I-labeled protein A, the filters were washed twice in 25 ml of 50 mM Tris, pH 7.4, 1 M NaCl, 5 mM EDTA, 0.25% gelatin, and 0.4% (wt/vol) sodium lauryl sarcosinate for a total of 12 to 18 h. The nitrocellulose filters were then air dried and exposed to Kodak XRP-1 X-ray film with a Du Pont Lightning-Plus intensifying screen at −80°C for 2 to 24 h.

RESULTS

Virion polypeptides. To study both the inter- and intrasubgroup immunological relationships of baculovirus structural polypeptides, 10 MNPVs, 3 SNPVs, and 4 GVs (listed in Table 1) were compared. AcMNPV ECV, which buds from the plasma membrane and is released into the medium in infected TN-368 cells at 8 to 48 h postinfection (32), was also included.

The structural polypeptides of these purified virions were compared by SDS-PAGE. Each baculovirus had a unique polypeptide pattern and contained from 15 to more than 25 polypeptides with molecular weights from about 12,000 to 120,000 (Fig. 1). A detailed analysis of the virion proteins of AcMNPV, *Rachiplusia ou* MNPV (RoMNPV), *Anticarsa gemmatalis* MNPV (AgMNPV), *H. armiger* MNPV (HaMNPV), TnSNPV, HzSNPV, TnGV, and *Spodoptera frugiperda* GV (SfGV) has been previously described (25). A comparison of AcMNPV ECV from infected TN-368 cells and AcMNPV purified from inclusion bodies has also been detailed previously (18, 30).

Protein blot RIA of baculovirus viral structural polypeptides. The procedure used to evaluate the immunological specificity of viral antisera was as follows. Purified virions (Table 1) were electrophoresed in SDS-polyacrylamide gels (as shown in Fig. 1) and transferred to two nitrocellulose filters by protein blotting. A 1:100 dilution of AcMNPV, PdMNPV, TnSNPV, HzSNPV, or TnGV antiserum was then reacted with the two virion polypeptide nitrocellulose filters. In all cases, similar immunological reactions were observed with a particular antiserum on duplicate polypeptide filters, with only minor quantitative differences being observed. We have reacted AcMNPV polypeptides by protein blot RIA more than a dozen times and have observed essentially the same immunological reactions as those seen in Fig. 2 (for example, compare the AcMNPV polypeptides that reacted with AcMNPV antiserum in Fig. 2 and 5). No reactions were observed with sera from nonimmunized rabbits (data not shown).

Radioimmunological reactions have been observed that do not align with Coomassie brilliant blue-stained polypeptides, e.g., AcMNPV antiserum reaction with an AcMNPV 16,000-molecular-weight polypeptide. Apparently, the protein blot RIA technique will detect antigens that are very low in concentration.

Immunological specificity of AcMNPV and PdMNPV antisera. Antiserum prepared to purified AcMNPV virions isolated from inclusion bodies was assayed against baculovirus virion polypeptides. AcMNPV antiserum recognized antigenic determinants on about 22 AcMNPV virion polypeptides (Fig. 2). This antiserum reacted with most of the major and many minor AcMNPV virus polypeptides. For example, a polypeptide of 41,000 molecular weight, a major capsid polypeptide (25), reacted strongly with homologous virion antiserum. An exception was a major capsid polypeptide of 18,500 molecular weight which has been shown by Tweeten et al. (28) to be an extremely basic protein. Although not well separated on this gel from two strongly reacting polypeptides of 16,000 and 19,000 molecular weight, this 18,500-dalton polypeptide reacted either weakly or not at all with AcMNPV antiserum (Fig. 2). Better separation of low-molecular-weight proteins was obtained on higher-percentage polyacrylamide gels, and the 18,500-dalton AcMNPV virion protein did not react with AcMNPV virion antiserum. AcMNPV antiserum also reacted strongly with four AcMNPV ECV polypeptides with molecular weights of 41,000, 64,000, 75,000, and 90,000 and weakly with a minor polypeptide of about 50,000 (Fig. 2). Table 2 summarizes remaining cross-reactions observed with AcMNPV antiserum in Fig. 2 and 3. Fifteen virion polypeptides of RoMNPV, a baculovirus closely related to AcMNPV (19), also cross-reacted with AcMNPV antiserum (Fig. 2). AcMNPV antiserum cross-reacted strongly with the 43,000-dalton AgMNPV and weakly with a minor polypeptide of 26,000 daltons. *Orgyia pseudotsugata* MNPV (OpMNPV), *Choristoneura fumiferana* MNPV (CfMNPV), TnSNPV, and HzSNPV virion

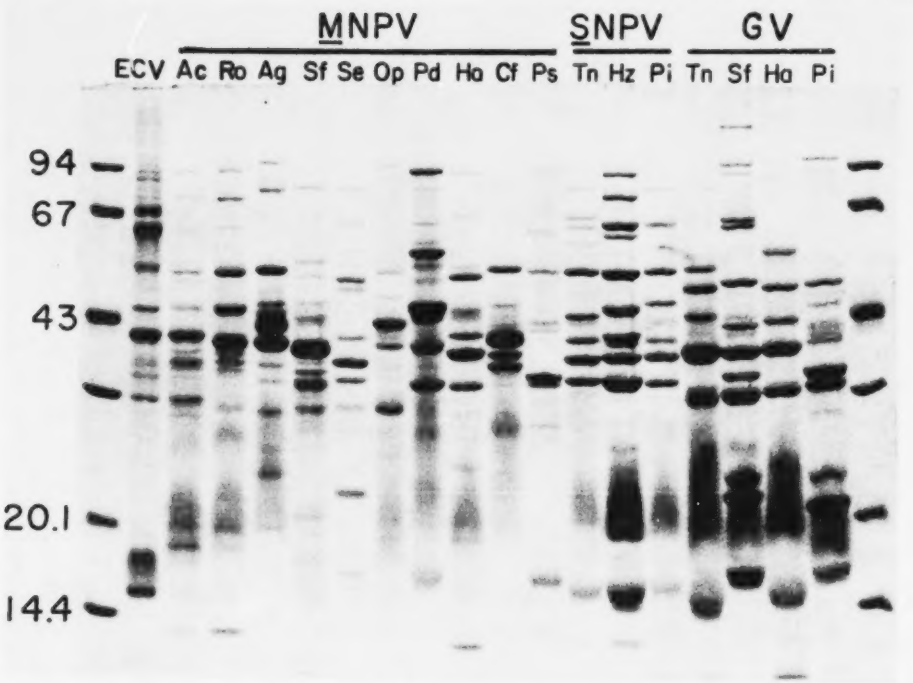

FIG. 1. *PAGE of the virion polypeptides of the baculoviruses listed in Table 1. About 30 μg of protein of each purified virus was disrupted, electrophoresed in 10% polyacrylamide, and stained as described in the text. Each preparation is abbreviated as described in Table 1. Protein molecular weight standards were electrophoresed in the first and last slots, and the molecular weights × 10^3 are indicated.*

polypeptides also cross-reacted with AcMNPV antiserum (Fig. 2 and 3).

Antiserum prepared to PdMNPV virions reacted to seven PdMNPV polypeptides (Fig. 2). Antigenic cross-reactions were also observed with the polypeptides of four other MNPVs and all three SNPVs (Table 2). No immunological relatedness was detected between either AcMNPV or PdMNPV antisera and the virion polypeptides of the four GVs.

Immunological specificity of TnSNPV and HzSNPV antisera. Antiserum prepared to TnSNPV virions reacted with only two TnSNPV polypeptides, of 39,000 and 34,000 molecular weight (Fig. 2). TnSNPV antiserum cross-reacted strongly with an HzSNPV 39,000 dalton polypeptide, a *Pseudoplusia includens* SNPV (PiSNPV) 39,000 dalton polypeptide, *S. exigua* (SeMNPV) 37,000- and 25,000-dalton polypeptides, a PiGV 17,000-dalton polypeptide, and weakly with SfMNPV 39,000- and HaGV 33,000-dalton polypeptides (Fig. 2 and 3).

Antiserum prepared to HzSNPV virions reacted with determinants on seven HzSNPV polypeptides (Fig. 3). Immunological cross-reactions were observed with PdMNPV 60,000- and SfGV 32,000- and 30,000-molecular-weight polypeptides.

Immunological specificity of TnGV antiserum. TnGV virion antiserum reacted with seven TnGV polypeptides (Fig. 3). The immunological reactions to two high-molecular-weight polypeptides of 120,000 and 150,000 may have been to protein aggregates of one or more smaller polypeptides (25). TnGV antiserum cross-reacted immunologically with several SfGV and HaGV polypeptides of 25,000 and 32,000 and weakly with PiGV 16,000- and HaMNPV 39,000-molecular-weight polypeptides. AcMNPV and RoMNPV polypeptides of 90,000 and 91,000 molecular weight, respectively, also had antigenic determinants recognized by antibodies to TnGV virions (Fig. 3). In a subsequent experiment, the immunological reaction of TnGV antiserum to an AcMNPV 90,000-dalton polypeptide was more intense than shown here. TnGV virion antiserum did not react with virion polypeptides of any SNPV.

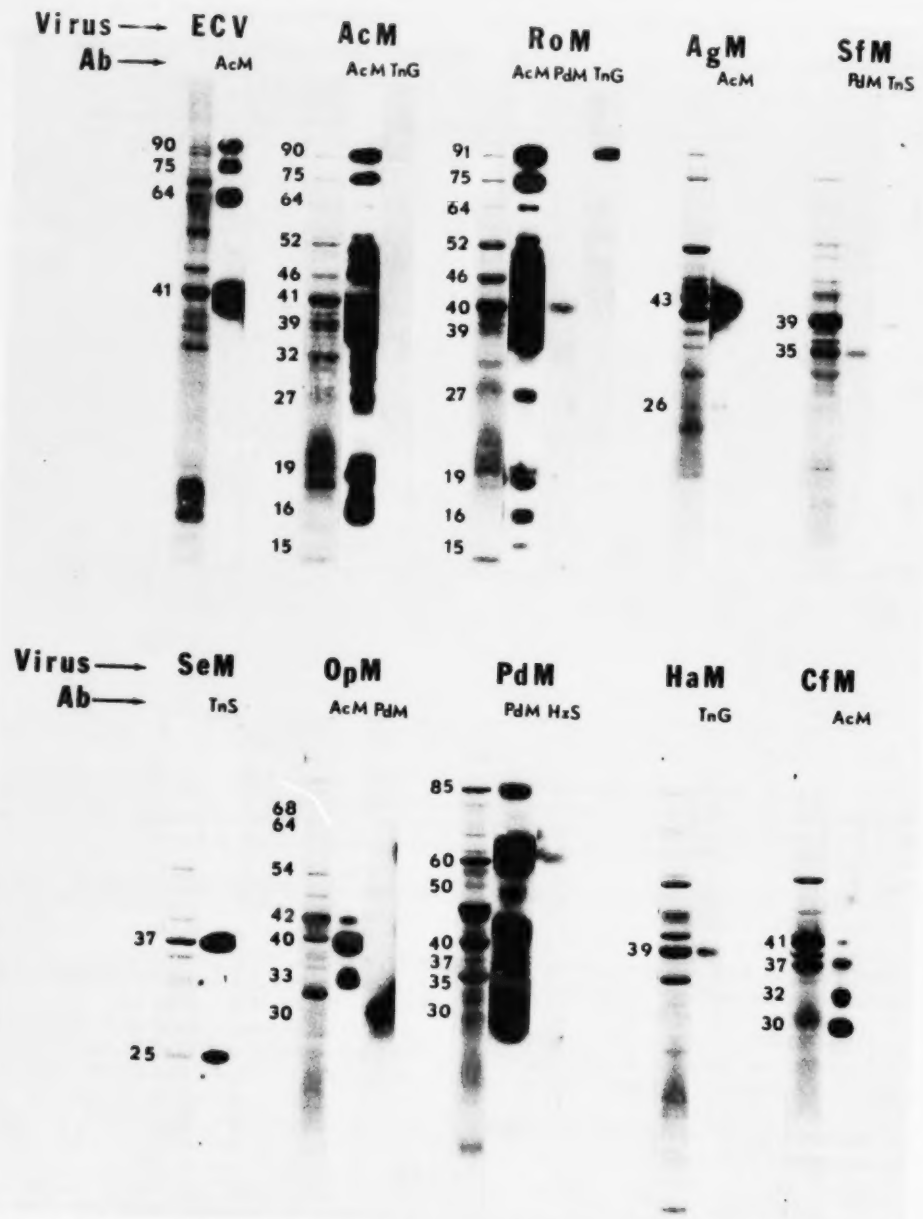
Virus → ECV AcM AgM SfM
Ab → AcM AcM TnG AcM PdM TnG AcM PdM TnS
90 75 64 41 52 46 41 39 32 27 19 16 15 91 40 43 26 35
Virus → SeM OpM PdM HaM CfM
Ab → TnS AcM PdM PdM HzS TnG AcM
68 64 54 42 37 33 30 25 85 60 50 40 37 35 39 41 32

FIG. 2. *Protein blot RIA analysis of the virion polypeptides of AcMNPV ECV and most of the MNPVs listed in Table 1. AcMNPV ECV, AcMNPV (AcM), RoMNPV (RoM), AgMNPV (AgM), SfMNPV (SfM), SeMNPV (SeM), OpMNPV (OpM), PdMNPV (PdM), HaMNPV (HaM), and CfMNPV (CfM) virion polypeptides were electrophoresed in 10% polyacrylamide, transferred to nitrocellulose filters, and reacted with a 1:100 dilution of antisera to AcMNPV, PdMNPV, TnSNPV, HzSNPV, and TnGV virions. Filters were incubated with ^{125}I-labeled protein A, washed, and autoradiographed for 12 h at −80°C as described in the text. The immunological reactions observed with the polypeptides of each virus and the five antisera tested are displayed as follows. In the first tract on the left is a Coomassie brilliant blue-stained preparation of the virion polypeptides. In adjacent tracts are the autoradiographs of the radioimmunological reactions observed. The antiserum used is labeled above each tract. The molecular weights × 10^3 of most of the immunological reactions are listed to the left of each virus preparation. Antisera which did not react with the virion polypeptides of a particular virus are not shown (for example, AcMNPV ECV, when tested against the five antisera used in this study, reacted with only the antiserum to AcMNPV virus).*

TABLE 2. *Common antigenic determinants on baculovirus virion polypeptides*

Antiserum	Cross-reactivity with given antigen[a]																	
	MNPV										SNPV			GV				Ac ECV
	Ac	Ro	Ag	Sf	Se	Op	Pd	Ha	Cf	Ps	Tn	Hz	Pi	Tn	SF	Ha	Pi	
AcMNPV	22	15	2	–	–	6	–	–	4	–	1	1	–	–	–	–	–	5
PdMNPV	–	1	–	1	–	1	7	–	–	1	2	2	2	–	–	–	–	–
TnSNPV	–	–	1	2	–	1	–	–	–	–	2	1	1	–	–	1	1	–
HzSNPV	–	–	–	–	–	–	1	–	–	–	–	7	–	–	2	1	–	–
TnGV	1	1	–	–	–	–	–	1	–	–	–	–	–	7	6	2	1	–

[a] Number of polypeptides that reacted with a specific virion antiserum. –, No detectable reaction. The molecular weights of the virion polypeptides that reacted can be obtained from Fig. 2 and 3. Antigens used were baculovirus virion polypeptides which had been electrophoretically separated in polyacrylamide gels and transferred to nitrocellulose filters. Each baculovirus is abbreviated as described in Table 1.

Immunological specificity of AcMNPV and HzSNPV polyhedrin antisera. The purified inclusion bodies of 16 baculovirus disrupted in alkali, and the soluble polyhedrin and granulin proteins were separated from virions and undissolved material by differential centrifugation. Portions of each preparation were electrophoresed in two SDS-polyacrylamide gels. One gel was stained for protein (Fig. 4), and the polypeptides in the other gel were transferred to two nitrocellulose filters and analyzed by protein blot RIA. Polyhedrin and granulin preparations were composed of a single major polypeptide with a molecular weight ranging from about 27,000 to 34,000. Low-molecular-weight polypeptides were also present as a result of incomplete inactivation of a protease associated with baculovirus inclusion bodies (24). Antisera to purified AcMNPV and HzSNPV polyhedrins cross-reacted with all of the polyhedrin and granulins when assayed by protein blot (Fig. 4). In addition, several lower-molecular-weight polypeptides polyhedrin and granulin proteolytic peptides also had antigenic determinants recognized by these antisera. However, not all of the polyhedrin and granulin proteolytic peptides had antigenic determinants that cross-reacted equally with both antisera. For example, several SeMNPV and PdMNPV proteolytic peptides polyhedrin cross-reacted strongly with HzSNPV polyhedrin antiserum but not with AcMNPV polyhedrin antiserum (Fig. 4).

Protein blot RIA of AcMNPV polyhedrin with homologous and virion polypeptides with homologous and heterologous antisera. AcMNPV inclusion bodies and AcMNPV virions purified from the same apbodies each had a polypeptide of the AcMNPV parent molecular weight (33,000) as whether the polyhedrin (Fig. 5). To determine we determivirion polypeptides shared antigeni whether ponants with AcMNPV polyhedrin or w to any virlyhedrin was immunologically linked preparation ion polypeptides, each polypeptide was tested by protein blot RIA with AcMNPV virion and polyhedrin antisera. Polyhedrin antigenic determinants were not detected on the virion 33,000-molecular-weight polypeptide (Fig. 5). A minor cross-reaction was observed with a lower-molecular-weight AcMNPV virion polypeptide (this reaction has not been observed in subsequent experiments). Consistent with these results was the lack of immunological relatedness between AcMNPV polyhedrin and AcMNPV virion antiserum (Fig. 5).

In a similar experiment, HzSNPV virion antiserum did not react with HzSNPV polyhedrin, and HzSNPV polyhedrin antiserum did not react with HzSNPV virion proteins (not shown).

DISCUSSION

The RIA used in this report (protein blot RIA) was essentially as described by Renart et al. (15) with two important modifications of Bowen et al. (2), who (i) developed a simple and efficient method for transferring electrophoretically separated polypeptides from polyacrylamide gels to nitrocellulose filters and (ii) used procedures to partially renature the polypeptides before transfer to the filters.

The extent to which baculovirus virion polypeptides were renatured and the actual number of antigenic determinants detected relative to the total that could be recognized by the antisera are now known. However, in preliminary experiments in which no attempt was made to renature the proteins before transfer and the electrophoretic transfer method Towbin et al. (27) was used, fewer AcMNPV virion polypeptides reacted with AcMNPV virion antisera than reported here (G. E. Smith and M. D. Summers, unpublished data). It is likely that not all of the antigenic determinants on the virion proteins were detected by protein blot RIA. Conformational determinants, especially those specified by the physical association of two different virion polypeptides, would be especially difficult to

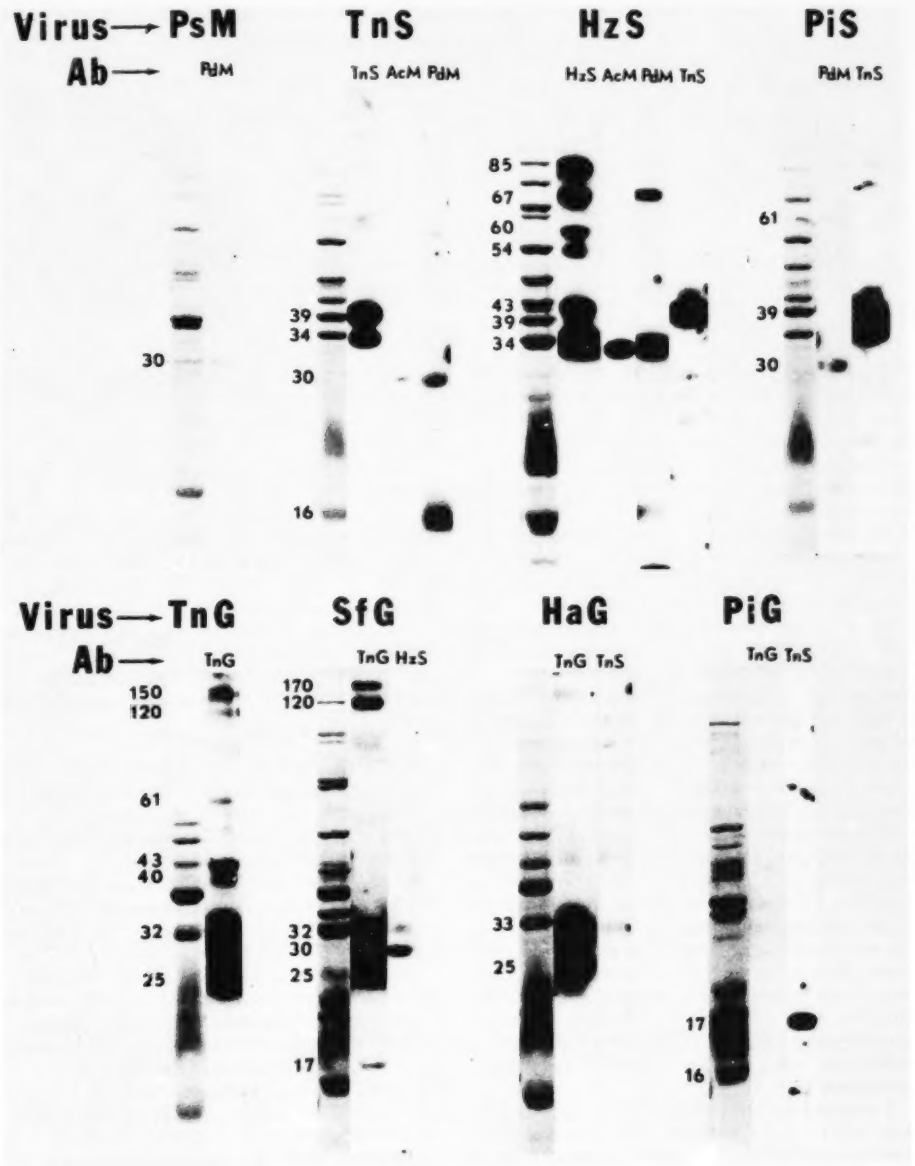

FIG. 3. *Protein blot RIA analysis of the virion polypeptides of the PsMNPVs, SNPVs, and GVs listed in Table 1. PsMNPV (PsM), TnSNPV (TnS), HzSNPV (HzS), PiSNPV (PiS), TnGV (TnG), SfGV (SfG), HaGV (HaG), and PiGV (PiG) virion polypeptides were electrophoresed in 10% polyacrylamide, transferred to nitrocellulose filters, and reacted ith 1:100 dilutions of antisera to AcMNPV, PdMNPV, TnSNPV, HzSNPV, and TnGV virions. Filters were incubated with ^{125}I-labeled protein A, washed, and autoradiographed for 12 h at −80°C as described in the text. The immunological reactions observed with the polypeptides of each virus and the five antisera tested are displayed as described in Fig. 2.*

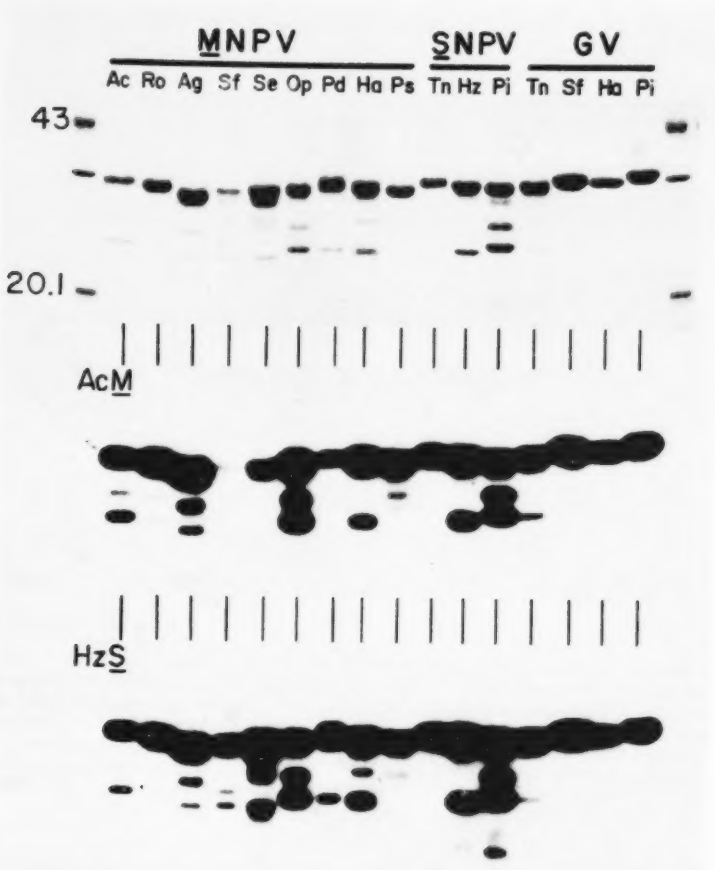

FIG. 4. *Protein blot RIA analysis of polyhedrins and granulins isolated from the baculoviruses listed in Table 1 (CfMNPV polyhedrin is not compared in this study). (Top panel) Polyhedrin and granulin preparations were electrophoresed in 10% polyacrylamide and stained with Coomassie brilliant blue. Protein standards were run in the first and last slots, and the molecular weights 10^3 are indicated. (Center and bottom panels) Autoradiogram of the polyhedrin and granulin preparations after protein blot RIA analysis with a 1:400 dilution of antiserum to AcMNPV polyhedrin and a 1:50 dilution of antiserum to HzSNPV polyhedrin, respectively. Autoradiographic exposures were for 2 h at −80°C.*

recover after SDS-PAGE. It is important, therefore, to interpret negative reactions between bacuolvirus polypeptides and viral antisera as only a lack of detectable immunological relatedness.

We have demonstrated in this study that baculovirus MNPVs, SNPVs, and GVs are immunologically related. The molecular weights of virion polypeptides with cross-reacting antigenic determinants were also determined. Harrap et al. (6) observed that virions from three MNPVs of *Spodoptera* have several cross-reacting antigens. One of these species, SfMNPV, was included in the present study. SfMNPV 39,000-dalton polypeptide, a major nucleocapsid polypeptide (6), reacted with TnSNPV virion antiserum. TnSNPV antiserum also reacted with a major 37,000-molecular-weight SeMNPV polypeptide. It is likely that this polypeptide is also a major nucleocapsid protein.

McCarthy and Lambiase (13) found, using immunodiffusion, an immunological reaction between TnSNPV nucleocapsid antiserum and AcMNPV virions. We have detected a minor

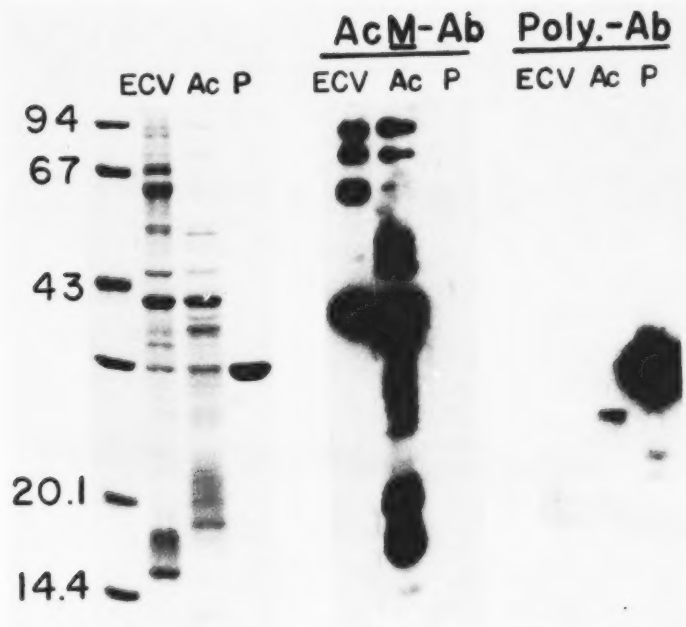

FIG. 5. *Protein blot RIA analysis of AcMNPV ECV and AcMNPV virion polypeptides and of AcMNPV polyhedrin. AcMNPV ECV and AcMNPV (Ac) virion polypeptides and AcMNPV polyhedrin (P) were electrophoresed in 10% polyacrylamide and stained with Coomassie brilliant blue (left panel). Protein molecular weight standards were electrophoresed in an adjacent tract, and the molecular weights × 10^3 are indicated. AcMNPV ECV and AcMNPV virion polypeptides and AcMNPV polyhedrin were electrophoresed on the same gel, transferred to two nitrocellulose filters, and reacted with a 1:100 dilution of AcMNPV virion antiserum (AcM-Ab) and a 1:400 dilution of AcMNPV polyhedrin antiserum (Poly-Ab). Autoradiographic exposures were for 12 h at −80°C.*

TnSNPV 26,000-molecular-weight polypeptide that reacts with AcMNPV antiserum. Unfortunately, McCarthy and Lambiase (13) did not characterize their TnSNPV nucleocapsid preparation by polyacrylamide gel analysis. Thus, it is not known whether the 26,000-molecular-weight polypeptide was also present in their TnSNPV nucleocapsid preparation. In that same study, TnSNPV nucleocapsid antiserum had a reaction identical to that of TnSNPV and PiSNPV virions. Similar results were also obtained by Pritchett et al. (14) and Singh et al. (17) in studies in which antisera were prepared against intact and ultrasonically treated virions, respectively. Our results were similar to these in that TnSNPV antiserum prepared against intact virions reacted with a 39,000-molecular-weight TnSNPV virion polypeptide and a similar-sized PiSNPV virion polypeptide.

We do not know why TnSNPV antiserum reacted with only two TnSNPV virion polypeptides whereas, for example, AcMNPV antiserum reacted with many homologous virion polypeptides. Since we have prepared each virion antiserum in only a few rabbits, there are insufficient data to speculate about this observation. However, disruption of TnSNPV virions before immunization might be expected to expose more viral structural antigens. We have obtained preliminary results (Knell, Smith, and Summers, manuscript in preparation) with TnSNPV and several other viruses that support this conclusion.

Pritchett et al. (14) reported that AcMNPV virions are not immunologically related to HaMNPV, HzSNPV, TnSNPV, and PiSNPV when compared by immunodiffusion. Using a more sensitive technique, protein blot RIA, we detected a minor cross-reaction with AcMNPV virion antiserum and a 34,000-molecular-weight HzSNPV capsid polypeptide.

In general, subgroup A baculoviruses have

interspecies antigenic determinants both between and among MNPVs and SNPVs. Therefore, the immunological data, which suggest that MNPVs and SNPVs may be genetically related, support classifying these two distinct morphological types into the same virus subgroup.

To our knowledge, there have been no reports of purified GV virions being immunologically related to NPVs. McCarthy and Lambiase (13) did not detect any immunological reaction between AcMNPV and TnSNPV nucleocapsid antisera and TnGV virions. In this report, we showed that subgroup B baculoviruses (GVs) were immunologically related to several subgroup A viruses. For example, TnGV virion antiserum reacted with AcMNPV, RoMNPV, and HaMNPV virion polypeptides of 90,000, 91,000, and 39,000 molecular weights, respectively. TnGV, AcMNPV, and RoMNPV will all replicate in the same insect host, *T. ni*. Thus, it was possible that TnGV was contaminated with, for example, RoMNPV. However, if this were true, RoMNPV antibodies would have been present in TnGV virion antiserum. Another possibility would be that the RoMNPV virion polypeptides transferred to nitrocellulose filters were contaminated with TnGV polypeptides. One advantage using protein blot RIA was that these and similar problems relating to cross-contamination of antigens could be eliminated by a careful analysis of the data. Specifically, the reaction of TnGV virion antibodies with RoMNPV 91,000-molecular-weight polypeptide was not due to contamination by RoMNPV virion antibodies. Such a contamination would have resulted in many of the major RoMNPV virion polypeptides reacting with the contaminating antibodies. Similarly, the RoMNPV virion polypeptides that were transferred to nitrocellulose were not contaminated with TnGV virion polypeptides. If this had been the case, TnGV virus antiserum would have reacted to the contaminating homologous antigens and not to RoMNPV 91,000-molecular-weight polypeptide.

An interesting result of this study was the difference in the reaction of AcMNPV virion antiserum to AcMNPV ECV and homologous viral polypeptides. Only four ECV polypeptides reacted strongly against antiserum prepared against AcMNPV virus isolated from inclusion bodies. In contrast, about 22 homologous AcMNPV virion polypeptides reacted with this same antiserum. This suggests that many of the virion structural antigens present on AcMNPV occluded virions (those assembled and occluded in the nucleus of infected cells) were not present on AcMNPV ECV. These differences between the structural antigens might, in part, be a result of the AcMNPV occluded virions obtaining their envelopes by de novo synthesis, whereas AcMNPV ECV acquires an envelope by budding through the plasma membrane.

All of the baculoviruses compared in this study have one or more major virion polypeptides with a molecular weight within the range of 33,000 to 43,000. Previous reports have shown that one of these is a major capsid polypeptide in AcMNPV, RoMNPV, AgMNPV, HzSNPV, and TnGV (25), SfMNPV (16), and PiGV (28). Many of the interspecies immunological cross-reactivities observed in this report involved major polypeptides having molecular weights also within the range of 33,000 to 43,000. For example, an HzSNPV capsid polypeptide of 34,000 had an antigenic determinant(s) recognized by AcMNPV, PdMNPV, and HzSNPV virion antibodies. TnSNPV virion antiserum cross-reacted with an SfMNPV 40,000-molecular-weight capsid polypeptide. Similar interspecies cross-reactivities were also observed with other major (and possibly capsid) polypeptides. By using interspecies radioimmunological methods, it has been possible to demonstrate (1) that all known mammalian type C viruses have interspecies determinants common to the major internal structural protein. In contrast, the antigenic sites on the envelope glycoproteins of these viruses are less well conserved (19). The present report provides preliminary data suggesting that antigenic sites on capsid polypeptides of baculoviruses may also be more conserved than determinants on the viral envelope proteins. One possible explanation for why internal proteins may have evolved more slowly than envelope proteins is that the viral capsid is not exposed to the selective pressures imposed by the direct interaction of the virion envelope with host cell membranes. Antisera to purified capsid polypeptides or monoclonal antibodies to capsid antigenic determinants are needed to determine whether capsid proteins share interspecies antigenic determinants.

All polyhedrins and granulins assayed were shown to contain antigenic sites recognized by AcMNPV and HzSNPV polyhedrin antisera. These cross-reacting determinants were present on the subunit polypeptides of 27,000 to 34,000 molecular weight. In previous studies (23), the possibility that immunological cross-reaction were due to determinants on higher-molecular-weight protein complexes of polyhedrin and granulin monomers or to new determinants on lower-molecular-weight proteolytic peptides could not be eliminated. Using protein blot RIA analysis, we showed that cross-reacting polyhedrin and granulin determinants were present on the major subunit polypeptides.

The differential cross-reactivities of AcMNPV

and HzMNPV polyhedrin antisera to heterologous proteolytic peptides suggest that these two antisera were reacting to different sets of cross-reacting determinants. It has also been shown, by using competitive RIA, that the avidity of polyhedrin and granulin for heterologous antibodies varies markedly (23). These results and those presented here indicate that interspecies antigenic determinants are not identical on polyhedrins and granulins but are composed of a spectrum of related determinants.

To relate immunological cross-reactions among baculoviruses to genetic similarity, interspecies antigenic determinants must be on viral translational products. The polyhedrins of AcMNPV and RoMNPV have been shown to be viral gene products (19, 26, 29). There is evidence that AcMNPV 90,000- and 41,000-molecular-weight polypeptides and RoMNPV 91,000- and 40,000-molecular-weight polypeptides are also virus specified (26). Thus, the immunological cross-reactions observed with these polypeptides probably reflect common nucleotide sequence homologies. We can conclude from these results that the immunological cross-reactivities detected among baculoviruses in this report provide good evidence that many subgroup A and B baculoviruses are genetically related. DNA homology studies with these same viruses (Smith and Summers, manuscript in preparation) confirm this conclusion.

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